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(54) **ACTIVATORS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR**

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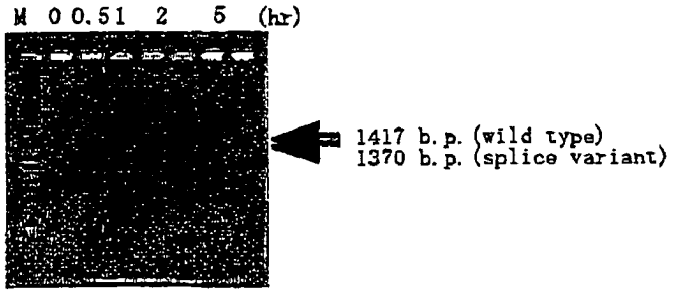
(57) **ABSTRACT**

Activators of peroxisome proliferator-activated receptors comprising a polyprenyl compound, preferably (2E,4E,6E, 10E)-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid, as an active ingredient, and medicaments for preventive and/or therapeutic treatment of hyperlipidemia, non-insulin dependent diabetes mellitus or the like comprising a polyprenyl compound as an active ingredient.

Fig.1



M : Marker

Fig.2

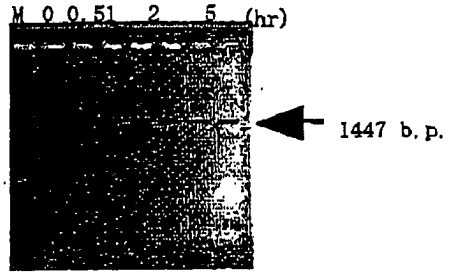


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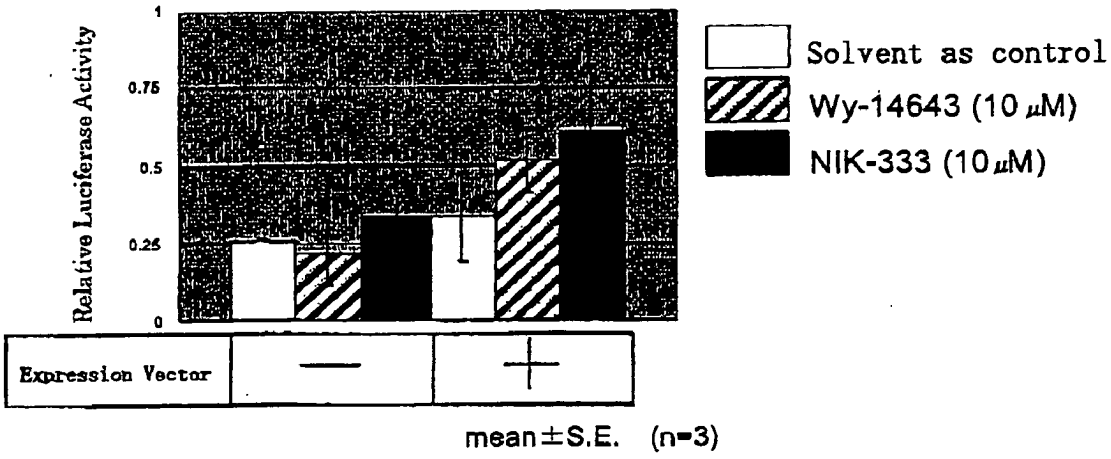
Fig.3



mean±S.E. (n=3)

ACTIVATORS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR

TECHNICAL FIELD

[0001] The present invention relates to an activator of peroxisome proliferator-activated receptors (abbreviated sometimes as "PPAR" in the specification).

BACKGROUND ART

[0002] Patients with hyperlipidemia or diabetes are estimated currently as 10 million or more in total in our country, and the number has been steadily increasing. Many of patients with diabetes suffer from non-insulin dependent diabetes mellitus, which is characterized by a pathological condition presenting hyperglycemia is resistant to the insulin action. Further, symptoms such as hyperinsulinemia, hypo HDL cholesterolemia, hypertension, and obesity most frequently occur with hyperlipidemia and diabetes, which raises clinical problems. In recent years, such pathological conditions presenting these multiple symptoms are referred to as Syndrome X, and considered as one of severe diseases (Reference: Diabetes, 37, 1595-1607 (1988)).

[0003] As medicaments for therapeutic treatment of these diseases, clofibrate derivatives including clofibrate as a typical example, thiazolidine derivatives including pioglitazone and troglitazone as typical examples and the like have been used. The clofibrate derivatives have an activating action on PPARα (Reference: Nature, 347, 645-650 (1990)) and are considered to improve lipid metabolism through the aid of fatty acid β-oxidation enzymes in the liver. The thiazolidine derivatives have an activating action on PPARγ (Reference: J. Biol. Chem., 270, 112953-112956 (1995)) and are considered to ameliorate insulin resistance and thereby lower a blood sugar level (Reference: Diabetes, 45, 1661-1669 (1996)).

[0004] However, PPAR agonists are reported to generally have adverse effects such as liver function failure, and accordingly, a patient with liver function failure contraindicates the use of troglitazone, one of the PPARγ agonists (Reference: Rinsho Iyaku, 14, 461-466 (1998)), and the sale of said drug was currently discontinued.

[0005] As described above, medicaments having the PPAR activation activity are useful as therapeutic agents for hyperlipidemia and diabetes. However, since they have various adverse effects, medicaments activating PPAR have been desired which have reduced adverse effects.

[0006] (2E,4E,6E,10E)-3,7,11,15-Tetramethyl-2,4,6,10, 14-hexadecapentaenoic acid (Development Code: "NIK-333"), one of polyprenyl compounds, is known to have affinities for retinoic acid binding proteins and retinoic acid receptors and to have actions of inducing differentiation and apoptosis in hepatocellular carcinoma. Clinically, NIK-333 significantly inhibited recurrence of hepatoma after radical treatment of hepatoma by long-term administration for one year, and thus its action of suppressing recurrence of hepatoma was suggested. Further, NIK-333 is proved to be a safe drug, because almost no liver function failure or almost no other adverse effect, generally accompanied with retinoids, was observed during the administration (Reference: N. Eng. J. Med., 334, 1561-1567 (1996)).

[0007] However, it has not been known that a polyprenyl compound activates PPAR.

DISCLOSURE OF THE INVENTION

[0008] Accordingly, an object of the present invention is to provide PPAR activators with reduced adverse effects.

[0009] The inventors of the present invention conducted various researches to find PPAR activators. As a result, they found that polyprenyl compounds induced expression of PPARα and PPARγ mRNAs and that the compounds had ligand activity for PPARα. On the basis of these results, they found that the polyprenyl compounds activated PPAR, and as a result of further researches, they achieved the present invention.

[0010] The present invention thus provides an activator of peroxisome proliferator-activated receptor (PPAR) comprising a polyprenyl compound as an active ingredient. The present invention further provides a medicament for preventive and/or therapeutic treatment of hyperlipidemia or non-insulin dependent diabetes mellitus which comprises a polyprenyl compound as an active ingredient.

[0011] From another aspect, the present invention provides a use of a polyprenyl compound for manufacture of the aforementioned medicament; a methods for activating peroxisome proliferator-activated receptor (PPAR) in a mammal including human, which comprise a step of administering an effective amount of a polyprenyl compound to a mammal including human; and a method for preventive and/or therapeutic treatment of hyperlipidemia or non-insulin dependent diabetes mellitus, which comprise the step of administering a preventively and/or therapeutically effective amount of a polyprenyl compound to a mammal including human in need of such preventive and/or therapeutic treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **FIG. 1** shows expression of PPARα mRNA in cells treated with NIK-333 (wild type, splice variant).

[0013] **FIG. 2** shows expression of PPARγ 1 mRNA in cells treated with NIK-333.

[0014] **FIG. 3** shows ligand activity of NIK-333 or Wy-14643 for PPARα with introduction of a PPARα expression vector (+) and without introduction of the same (−).

BEST MODE FOR CARRYING OUT THE INVENTION

[0015] The whole disclosures of Japanese Patent Application No. 2000-122974 (filed on Apr. 24, 2000) are incorporated by reference in disclosures in the specification.

[0016] Among polyprenyl compounds used in the present invention, a particularly preferred compound includes (2E, 4E,6E,10E)-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid (NIK-333). Other examples of the polyprenyl compounds include conjugated polyprenylcarboxylic acids (polyprenoic acids) such as 3,7,11,15-tetramethyl-2,4,6,10, 14-hexadecapentaenoic acid and esters thereof described in Japanese Patent Publication (Kokoku) No. 63-34855 and the like.

[0017] The polyprenyl compounds used in the present invention can be synthesized by a known method (Japanese Patent Publication (Kokoku) No. 63-32058, J. Chem. Soc. (C), 2154 (1966)).

[0018] When the PPAR activator of the present invention or the medicament of the present invention for preventive and/or therapeutic treatment of hyperlipidemia or non-insulin dependent diabetes mellitus based on the PPAR activating action is used, a pharmaceutical composition comprising the polyprenyl compound can be generally prepared and administered via an appropriate administration route, i.e., oral or parenteral route. Examples of forms of the pharmaceutical composition suitable for oral administration include tablets, granules, capsules, soft capsules, pills, powders, solutions and the like. Examples of forms of the pharmaceutical composition suitable for parenteral administration include injections, suppositories and the like. These pharmaceutical compositions can be prepared by an ordinary method using a polyprenyl compound or a pharmacologically acceptable salt thereof and one or more kinds of ordinary pharmaceutically acceptable pharmaceutical carriers. Two or more kinds of polyprenyl compounds as active ingredients may be used in combination.

[0019] For example, for the medicaments suitable for oral administration, desired pharmaceutical compositions can be prepared by using, as pharmaceutical carriers, excipients such as lactose, glucose, corn starch, and sucrose, disintegrants such as carboxymethylcellulose calcium, and hydroxypropylcellulose, lubricants such as calcium stearate, magnesium stearate, talc, polyethylene glycol, and hydrogenated oil, binders such as hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose, polyvinyl alcohol, gelatin, and gum arabic, moistening agents such as glycerine and ethylene glycol, as well as surfactants, flavoring agents and the like as optionally required.

[0020] For the medicaments suitable for parenteral administration, diluents such as water, ethanol, glycerine, propylene glycol, polyethylene glycol, vegetable oil, agar, and gum tragacanth may be used as pharmaceutical carriers, as well as solubilizing agents, suspending agents, emulsifiers, buffers, isotonic agents, preservatives, soothing agents and the like may be used as optionally required.

[0021] The medicament of the present invention can be applied to diseases that are therapeutically and/or preventively treatable by PPAR activation, and the medicament can be used for mammals including human. Examples of PPAR that can be activated by the medicament of the present invention include PPARα or PPARγ as preferred targets. Examples of conditions to which the medicaments of the present invention can be preferably applied include non-insulin dependent diabetes mellitus and hyperlipidemia, as well as complications of these diseases, for example, hyperinsulinemia, hypo HDL cholesterolemia, hypertension, obesity and the like.

[0022] When the PPAR activator of the present invention or the medicament of the present invention for preventive and/or therapeutic treatment of hyperlipidemia or non-insulin dependent diabetes mellitus based on the PPAR activating action is used, doses are not particularly limited. For example, 1 to 2,000 mg, preferably 20 and 800 mg, can be administered per day for an adult for oral administration. For parenteral administration, doses may be in the range of 1 to 1,000 mg, preferably in the range of 10 to 100 mg. Desired preventive and/or therapeutic effects can be expected by administration of the aforementioned dose once to 3 times a day.

EXAMPLES

[0023] The present invention will be explained more specifically with reference to the following examples. However, the scope of the present invention is not limited to these examples.

Example 1

Expression of PPARα and PPARγ mRNA in Human Cell Line

[0024] Caco-2 cells (derived from colon cancer) as a human cell line were cultured at 37° C. in RPMI-1640 medium containing 10% fetal calf serum in the presence of 5% CO_2. The medium was then replaced with serum-free RPMI-1640 medium and the cells were cultured for 48 hours. In order to examine the effect of NIK-333, a solution of NIK-333 in ethanol was added at a final concentration of 10 μM. At 0, 0.5, 1, 2 and 5 hours after the addition, RNA was extracted to observe mRNA for PPARα, PPARγ 1 and PPARγ 2 by the RT-PCR method.

[0025] As a result, expression of PPARα mRNA was observed from 0.5 hour after the addition of NIK-333 (**FIG. 1**). Further, expression of PPARγ 1 mRNA was also observed from 0.5 hour after the addition of NIK-333 (**FIG. 2**), whilst expression of PPARγ 2 mRNA was not observed.

Example 2

Ligand Activity for PPARα

[0026] COS-7 cells, a cell line derived from monkey kidney, were cultured at 37° C. in DMEM medium containing 10% fetal calf serum in the presence of 5% CO_2. Then, expression vectors of RXRα (retinoic acid X receptor α) and PPAR a, and a reporter vector incorporated with PPRE (peroxisome proliferator-responsive element) as a PPAR-responsive element were cotransfected into the cells, and the cells were cultured for 24 hours. In order to examine the effect of NIK-333, a solution of NIK-333 or Wy-14643 (selective agonist of PPARα) in ethanol was added at a final concentration of 10 μM. After cultivation for 24 hours, the activity of firefly luciferase was measured. The measured values were represented as values standardized by using the renilla luciferase activity.

[0027] As shown in **FIG. 3**, NIK-333 and Wy-14643 failed to increase the luciferase activity when the PPARα expression vector was not introduced (−), whereas they increased the luciferase activity only when the PPARα expression vector was introduced (+). NIK-333 exhibited increasing action of ligand activity almost equivalent to the action of Wy-14643.

INDUSTRIAL APPLICABILITY

[0028] Polyprenyl compounds induce expression of PPARα and PPARγ and also have ligand activity for PPARα. Therefore, these compounds have a PPAR activation action and are useful for preventive and/or therapeutic treatment of hyperlipidemia or non-insulin dependent diabetes mellitus.

1-13. (canceled)

14. A medicament which comprises a polyprenyl compound as an active ingredient, wherein the medicament is associated with instructions to administer the medicament to

prevent or treat at least one of hyperlipidemia and non-insulin dependent diabetes mellitus.

15. The medicament of claim 14, wherein the polyprenyl compound comprises at least one of a polyprenylcarboxylic acid and an ester thereof.

16. The medicament of claim 14, wherein the polyprenyl compound comprises 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid.

17. A method of preventing or treating hyperlipidemia, wherein the method comprises administering a preventively or therapeutically effective amount of at least one polyprenyl compound to a patient in need thereof.

18. The method of claim 17, wherein the at least one polyprenyl compound comprises at least one of a polyprenylcarboxylic acid and an ester thereof.

19. The method of claim 18, wherein the at least one polyprenyl compound comprises 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid.

20. The method of claim 18, wherein the at least one polyprenyl compound comprises (2E,4E,6E,10E)-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid.

21. The method of claim 17, wherein the at least one polyprenyl compound is in an orally administrable form.

22. A method of preventing or treating non-insulin dependent diabetes mellitus, wherein the method comprises administering a preventively or therapeutically effective amount of at least one polyprenyl compound to a patient in need thereof.

23. The method of claim 22, wherein the at least one polyprenyl compound comprises at least one of a polyprenylcarboxylic acid and an ester thereof.

24. The method of claim 23, wherein the at least one polyprenyl compound comprises 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid.

25. The method of claim 23, wherein the at least one polyprenyl compound comprises (2E,4E,6E,10E)-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid.

26. The method of claim 22, wherein the at least one polyprenyl compound is in an orally administrable form.

27. A method of activating a peroxisome proliferator-activated receptor (PPAR), wherein the method comprises administering a PPAR activating amount of at least one polyprenyl compound to a patient in need of such activation.

28. The method of claim 27, wherein the at least one polyprenyl compound comprises at least one of a polyprenylcarboxylic acid and an ester thereof.

29. The method of claim 28, wherein the at least one polyprenyl compound comprises 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid.

30. The method of claim 28, wherein the at least one polyprenyl compound comprises (2E,4E,6E, 10E)-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid.

31. The method of claim 27, wherein the at least one polyprenyl compound is in an orally administrable form.

32. The method of claim 27, wherein the PPAR comprises PPARα.

33. The method of claim 27, wherein the PPAR comprises PPARγ.

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